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The Action of Phosphates in Sausage Products. I. Factors Affecting the Water Retention of Phosphate-Treated Ground Meat[a]

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THE PRESENT ARTICLE is the third of a series *(16, 17)* concerned with the chemistry and technology of sausage products, such as frankfurters and bologna, and the first of this series concerned with the effects of adding phosphates to meat. The emphasis given this work originated with observations of interest in the industry on possible advantages of adding phosphates to sausage formulas, and recognition of the inadequate knowledge presented by contradictory available reports on the results to be expected from their use.

The practice of adding the phosphates, disodium ortho-, sodium tripoly-, tetra and disodium pyro-, or sodium hexameta-, to pickles for curing pork cuts, such as hams and picnics, has recently become rather common and is accepted by the USDA Meat Inspection Branch *(18)*. This development occurred without the appearance of technical literature on processing and product characteristics; however, its general acceptance is *a priori* evidence that the additives improve one or more of such characteristics as firmness, moisture retention, and color of pork cuts.

Use of phosphate additives in sausage products has not been approved by the Meat Inspection Branch up to the present time. A limited amount of information on effects of phosphate additives in sausage, such as frankfurters and bologna, has been made available. Wilson *(19)* indicated that no beneficial effect was found in tests of several of the phosphates in commercial-type products. On the other hand, Frank *(3)* recently reported that tetrasodium pyrophosphate, but not other phosphates, improved frankfurters with respect to moisture retention. Others who report that phosphate additives can decrease cooking losses and fat separation include Heil *(7)* and Ellerkamp and Hannerland *(2)*. A recently published review by Morse *(13)* of some of the literature on phosphates indicated that there is some agreement, together with considerable speculation, on the properties and function of phosphates in meat. There has as yet been no attempt to report information relating to the effects of phosphates on color, texture, processing time and temperature, and other factors involved in sausage making.

Both laboratory studies and pilot plant investigation have been conducted in the present work to obtain an understanding of the complex relations existing between the properties of meat, curing agents, and processing methods. Results of a comprehensive study of processing and sausage quality that has been carried out in pilot plant work will be described in an additional paper. Background information obtained in the laboratory investigation of the effects of phosphates on meat, especially emphasizing factors affecting water retention, is reported in the present paper. It should be noted that the present paper does not indicate or recommend that increasing the water content of meat products is desirable.

A great deal of foundation information for our work was taken from reports of Grau *et al. (4)* and Möhler and Kiermeier *(8, 9, 10, 11)* and Hamm *(5)*. The results of Grau and of Möhler and Kiermeier *(8)* indicate that increasing the alkalinity of meat increases its ability to retain moisture. According to the latter authors, this explanation only partially accounts for the effects of the polyphosphates *(10)*. As the result of another investigation of the swelling produced by polyphosphates, Bendall *(1)* concluded that increased ionic strength produced the swelling observed in the use of orthophosphates and polyphosphates, except that pyrophosphates produced an additional or "specific" swelling. Bendall proposed that this effect possibly involves splitting actomyosin. The fact that pyrophosphates, under certain conditions, split actomyosin, yielding actin and myosin, has been adequately demonstrated by Straub *(15)*. Tetrasodium pyrophosphate has also been shown by Hasselbach and Schneider *(6)* to be effective in dissolving muscle protein. Its action, according to Mommaerts *(12)*, is markedly sensitive to temperature and the presence of magnesium. As will be discussed, some of the experiments described herein were designed to investigate the "specific" effect stressed by Bendall, since this effect would possibly be of importance. In a recently-published paper, Hamm *(5)* attributed phosphate effects partly to increasing meat pH and to salting action, and also, the importance of the binding of calcium and magnesium was stressed as an important factor affecting the action of the phosphates on meats.

Inasmuch as previous studies on water retention have not yielded consistent and conclusive evidence as to the relative importance of ionic strength, pH, time, and temperature, and of the importance of a "specific" effect and of calcium and magnesium binding, this investigation centered around the study of these factors. The additives studied were mainly mixtures of sodium chloride and pyrophosphates and, to a limited extent, mixtures of sodium chloride with the sodium orthophosphates. In the investigations of the mechanism of additives, calcium and magnesium chlorides and potassium iodide were added.

METHODS

The principal method employed in determining moisture retention was a modification of that described by Bendall *(1)*. This involved adding solutions of salts to meat in the propor-

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tion of 2 ml. of solution to 1 g. of meat and measuring unabsorbed solution after samples had been stored and centrifuged (Method A-C) and again after they had been heated (Method A-H). A smaller number of experiments was conducted using a method which involved determining the amount of moisture evaporated on heating meat treated with salt solutions in the proportion of 0.5 ml. of solution per gram of meat. Several exceptions are noted in the descriptions of the experiments; otherwise, the methods used are described in the following:

Method A-C: Sufficient frozen beef for an entire experiment was completely thawed, trimmed as free as possible of fat and connective tissue, ground through an ordinary food chopper, and thoroughly mixed. Twenty-g. (± .02 g.) portions of meat were placed in 60 ml.-capacity centrifuge tubes and 20 ml. of salt solution at *ca.* 32-41° F. (0.5° C.) was added to each tube by pipette. The solutions and the meat were thoroughly mixed with a stirring rod, after which the mixtures were allowed to stand for approximately 16 hours at 32° F. (0° C.) The tubes were then centrifuged for 15 minutes at 3300 r.p.m. in a Servall angle centrifuge. The volume of supernatant liquid which separated in each tube was determined by decanting the fluid into a 25 ml. graduated cylinder calibrated at 0.2 ml. intervals. The volume of the samples of the treated meat was calculated by subtracting the volume of the decanted supernatant liquid from the original total volume, assumed to be 40 ml. These averages of values, obtained in duplicate determinations, expressed as a percentage of the original volume of the meat which was assumed to be 20 ml. for this purpose, are termed "unheated" volumes.

Method A-H: The centrifuge tubes containing the swollen meat were placed in a water bath at 158° F. (70° C.) for 15 minutes. Any supernatant liquid released from the meat was immediately decanted and its volume measured as described above. The volume of the samples of heated meat was calculated by determining the sum of the volume of solution released on heating and the volume of solution decanted in following Method A-C, and subtracting this sum from 40 ml. The average of volumes of duplicate determinations was also expressed as percentages of the original volume of the meat (20 ml.) and termed the "heated" volume.

Fifteen samples from a single batch of ground beef treated with 1% $Na_4P_2O_7/Na_2H_2P_2O_7$-1.7% NaCl, pH 7.5, had a mean unheated volume of 150.1%, mean deviation, ± 1.18%, and a mean heated volume of 112.4%, mean deviation ± 2.06%.

Method B: A thoroughly mixed 300-g. batch of ground beef was divided into two 150-g. portions; 75 ml. of different solutions were added to the 150-g. portions at 32-41° F. (0-5° C.); the solutions and meat were thoroughly mixed; and the mixtures allowed to stand overnight at 32-41° F. (0-5° C.). After storage, the mixtures were again thoroughly stirred and six 30-g. (± .01 g.) portions of each batch were transferred to tared aluminum dishes. The dishes and contents were weighed and placed in a constant temperature oven equipped with a revolving shelf, at 167° F. (75° C.). After two hours of heating, the dishes and contents were removed, cooled one-half hour to room temperature, and weighed. From the data obtained, the moisture lost by evaporation was calculated; these losses are expressed in the following as percentages of the weight of the original samples (30 g.). The mean evaporation of samples representing each treatment was calculated and the significance of any differences between the two means was determined using the "t" test.

Preparation of solutions. The pyro- and orthophosphate solutions employed were titrated to adjust them with respect to pH value and, frequently, were also adjusted with respect to ionic strength. Freshly prepared solutions were always used. The concentrations of solutions of $Na_4P_2O_7/Na_2H_2P_2O_7$ and Na_2HPO_4/NaH_2PO_4 are expressed as the concentrations of the $Na_2H_2P_2O_7$ and NaH_2PO_4, respectively, that would be available on complete acidification.

Meat used. The meat used, except for rabbit meat employed in one series of experiments, was steer beef. The meat was separated from carcasses stored at 37° F. (2.8° C.) for 3-7 days after slaughter. The meat was wrapped in cellophane and placed in boxes holding either 25 or 50 pounds of meat. These were stored at 0° F. (—17.8° C.). Portions were cut from the blocks of beef with a bandsaw as needed and allowed to thaw overnight. Muscle sections which were relatively free of fat or connective tissue were removed for experimental use. The various storage periods during which the beef was frozen are indicated in the descriptions of experiments that follow.

EXPERIMENTAL AND RESULTS

Relation of unheated to heated volumes. Relation of unheated to heated volumes was investigated, using Methods A-C and A-H, respectively, on steer beef which had been frozen approximately 7 weeks. The treatments applied consisted of different solutions containing from 3.6 to 8.0% sodium chloride and different solutions of 1% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.7% NaCl, varying with respect to pH over the range 5.5 to 9.0. Under these conditions, increasing pH was accompanied by increasing ionic strength. The results of this experiment, represented by curves showing the relation between unheated and heated volumes, are shown in Figure 1.

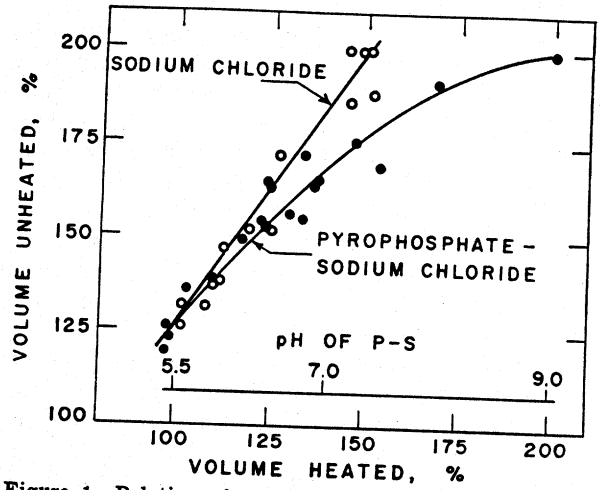

Figure 1. Relation of volumes of unheated to heated meat samples treated with sodium chloride (○) and pyrophosphate-sodium chloride, pH 5.5 to 9.0 (●).

The unheated and heated volumes of samples treated with sodium chloride solutions were found to be linearly related. Part of the curve expressing the relation of the unheated and heated volumes of samples treated with pyrophosphate-sodium chloride solutions of pH 7, or less, closely approached this straight line. On the other hand, the overall relation of heated and unheated volumes of samples treated with pyrophosphate-sodium chloride solutions was curvilinear, *i.e.*, as the alkalinity (and ionic strength) of the solutions applied to the meat increased, the tendency to retain moisture on heating increased. This result indicates that errors may be introduced by determining the relative merits of treatment with solutions of high pH on the basis of unheated volumes only, although for many purposes this determination alone is sufficient. It was noted in repetitions of the above experiment that, possibly owing to differences in beef, curves shown in Figure 1 were not exactly reproduced, although the slope was the same. From his investigation of this relationship, Bendall *(1)* reported that volumes of unheated and heated samples were linearly related for all of the more limited range of treatments studied.

Effect of varying temperature of storage. Three series, each consisting of 6 samples, were prepared, the samples in a series being treated either with (1) 1% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.7% NaCl, pH 7.5; (2) 1.42% Na_2HPO_4/NaH_2PO_4–1.7% NaCl, pH 7.0; or (3) 3.4% NaCl. Steer beef which had been frozen one week was used. Methods A-C and A-H were followed, except that pairs of samples from each series were stored at 32° F. (0° C.), 50° F. (10° C.), or 68° F. (20° C.). The volumes of the samples after storage at the 3 temperatures are shown in Figure 2. Results show that the volume of samples treated with pyrophosphate-sodium chloride and orthophosphate-sodium chloride markedly decrease as the temperature of storage increased. Varying the temperature had no similar effect on the volume of samples treated only with sodium chloride. It is not possible at present to propose a reliable explanation for the observed difference. These results indicate that maintaining temperatures approaching 32° F. (0° C.) may be of considerable practical importance in obtaining optimum moisture retention in using phosphates.

Effect of varying time of storage. Two series of samples were prepared; the samples in one series were treated with a solution of 1.0% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.7% NaCl, pH 6.8, and those in the other with a solution of 3.6% NaCl. Using steer beef which had been frozen 3 weeks, Method A-C was followed except that the samples were stored at 32° F. (0° C.) for different intervals ranging from 1 to 30 hours. Curves based on

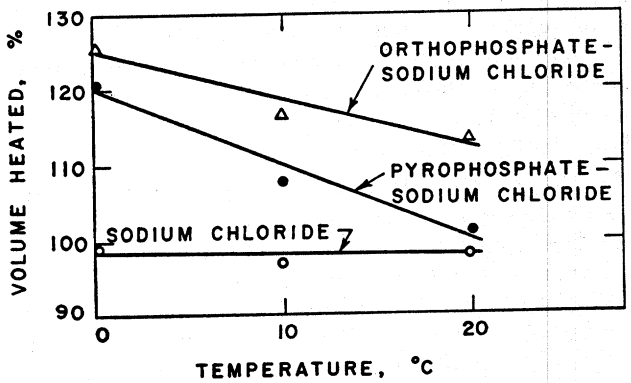

Figure 2. Effect on volumes of meat samples of treatments at different temperatures with orthophosphate-sodium chloride (△), pyrophosphate-sodium chloride (●) and sodium chloride (○).

the results, showing the relation between volumes and time in storage, and also between volumes, expressed as a percentage of the maximum attained (in 30 hours), and time in storage, are shown in Figure 3.

The slopes of the curves indicate that the volume of the samples in both series increased with increasing storage; after 16 hours of storage the volumes were only slightly less than those obtained after 30 hours of storage. Curves showing the relation between the volumes, expressed as percentage of maximum volume, and time of storage were similar, indicating that there was no fundamental difference in the rate at which the two treatments attained maximum effectiveness.

In another experiment it was found that treating meat that had been more thoroughly minced by grinding it through a 1/16-inch plate required approximately the same amount of time to become fully effective. The importance of the time factor has previously been observed in extracting muscle proteins; for example, 20-minute extractions separate myosin from actomyosin, although the same solution extracts actomyosin over a longer period of time *(6)*. The evidence showed that allowing sufficient time for the reaction(s), probably not in excess of 16 hours, is essential in obtaining a major portion of the potential moisture retention.

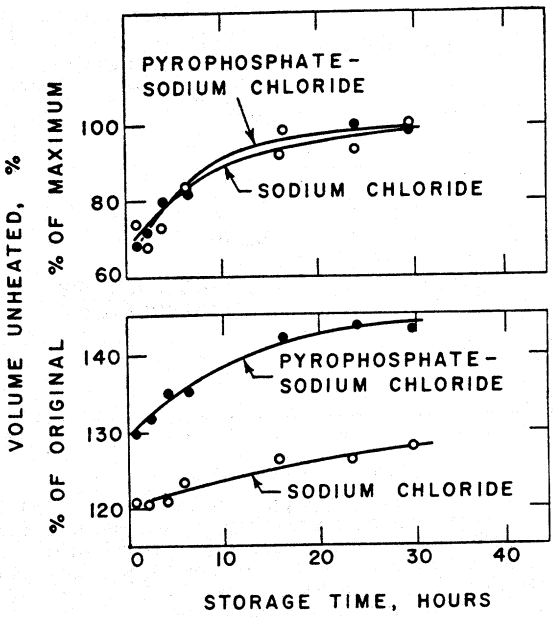

Figure 3. Effect of varying storage at 0° C. on volumes of meat samples treated with pyrophosphate-sodium chloride (●) and with sodium chloride (○).

The effects of pH and ionic strength.[b] Two series of samples were prepared; one series of samples was treated with solutions of 1% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.7% NaCl adjusted to pH values of 5.3, 5.5, 5.75, 6.0, 6.45, and 7.0, having ionic strength values of 0.305, 0.307, 0.312, 0.320, 0.331, and 0.349, respectively. The other series of samples was treated with 3.574, 3.613, 3.655, 3.755, 3.884, and 4.094% solutions of NaCl, the ionic strength values of these solutions corresponding with those of the solutions of pyrophosphate-sodium chloride. Using steer beef which had been frozen for one week, all samples were treated by Method A-C. The relation of the volumes (unheated) of samples to the ionic strength of the solutions applied is indicated by the curves shown in Figure 4. A curve is included which shows the volume of the samples treated with pyrophosphate-sodium chloride in relation to ionic strengths (μ) corrected for the buffering action of the meat. To calculate the latter values, it was assumed that the ionic strength values were the sum of the ionic strength of sodium chloride (1) directly added and (2) that formed during the titration to adjust the pH of the solutions, and the ionic strength of $Na_4P_2O_7/Na_2H_2P_2O_7$ at the pH (5.45 to 5.95) of the treated samples. Reduction of the pH of solutions in the

[b] The concentration of each ion (in gram-ions per 1000 g. solvent) is multiplied by the square of its valence: the sum of these quantities, divided by 2, equals ionic strength.

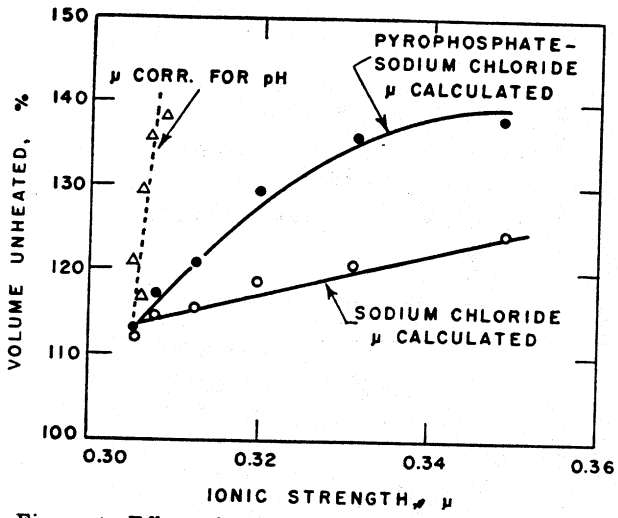

Figure 4. Effect of varying pH and ionic strength (μ) on volumes of meat samples treated with pyrophosphate-sodium chloride (●) and with sodium chloride (○) shown in relation to μ directly calculated. Also the volume of pyrophosphate-sodium chloride treated samples are shown with reference to μ corrected for the buffering action of the meat. (△).

presence of the meat sharply reduced ionic strength, since the ratio of the ionic strength of $Na_4P_2O_7$ to that of $Na_2H_2P_2O_7$ is 10 to 3. Similar calculations were made in the work reported by Bendall *(1)*.

Moisture retention of meat treated with sodium chloride was linearly related to the ionic strength of the solutions applied. The volumes of meat treated with pyrophosphate-sodium chloride solutions exceeded that of meat treated only with sodium chloride at any corresponding ionic strength; furthermore, the differences increased with increasing ionic strength and pH. The greater effect of the pyrophosphate-sodium chloride treatments appears to be attributable to the effect of the alkalinity of the pyrophosphate solutions. If the special calculations of ionic strength values described above are assumed to be valid, the effects of increasing pH appear even more pronounced, since the curve indicating the relation of volumes to ionic strength values modified by the meat shows that the volumes of pyrophosphate-sodium chloride treated samples increased markedly at nearly constant ionic strength (0.305 to 0.309). Other factors possibly involved in the effect of the pyrophosphates, such as binding of calcium and magnesium and a specific effect, do not appear to be of major importance, based on further observations discussed in the following.

Specificity. In a second experiment, solutions consisting of 1% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.7% NaCl, pH 7.5, 0.66 ionic strength, and 1.68% Na_2HPO_4/NaH_2PO_4-1.7% NaCl, pH 7.0, 0.66 ionic strength, were used to treat samples of muscle from freshly killed rabbits, treatments being applied after storage at 37° F. (2.8° C. for 2, 22, 24, 46, and 140 hours post mortem. Methods A-C and A-H were followed. The results, showing the heated volumes and the pH of treated samples, are given in Table 1.

Slightly larger volumes were obtained in treating fresh (stored 2 hours) muscle with pyrophosphate-sodium chloride than with orthophosphate-sodium chloride. On the other hand, the volume of muscles stored 22 or more hours was increased somewhat more by the treatment with orthophosphate-sodium chloride. One explanation of this is that the orthophosphate was more effective in buffering against the decrease in pH as the meat aged. The data show that the orthophosphate-sodium chloride treatment increased the pH of muscle stored 22 or more hours as much as 0.2 units more than did the pyrophosphate-sodium chloride treatment. The fact that the treatments with pyrophosphate-sodium chloride solutions were less effective than those with orthophosphate-sodium chloride, when applied to stored samples presumably containing considerable actomyosin, appears to indicate that the mode of action of the pyrophosphate did not involve any appreciable specific action, such as splitting actomyosin as Bendall *(1)* proposed. Otherwise, it would have exerted an equal or probably greater effect as compared with orthophosphate, which has not been reported to exert a splitting or specific action on actomyosin, except insofar as salts in general may possess some activity. Their buffering action, along with their effect in increasing ionic strength, appear to be principal factors determining the order of effectiveness of the pyro- and orthophosphate-sodium chloride combinations.

Marked effect achieved with potassium iodide. Employing Methods A-C and A-H, using steer beef which had been frozen for 20 weeks, a series of samples was treated with solutions containing 3.63% NaCl; 3.59% NaCl-0.10% KI; 3.26% NaCl-1.03% KI; 2.7% NaCl-2.57% KI; and 1.82% NaCl-5.15% KI, the ionic strength of all solutions being 0.62. The effect of varying the proportions of the salts on moisture retention, as indicated by the volume of heated samples after treatment with the various combinations, is shown in Figure 5. The results show that the addition of potassium iodide markedly increased the retention of moisture, the effect increasing as the concentration of KI increased.

TABLE 1
Volume and pH of fresh and stored rabbit muscle treated with pyro- and orthophosphates and sodium chloride

Rabbit number		1	2		3		4	
Storage, hours		2	22	46	2	24	2	140
Treatment								
Pyrophosphate-sodium chloride[1]	volume, %	146	122	153	130	113	134	120
	pH	6.55	6.60	6.70	6.45	6.60	6.60	6.30
Orthophosphate-sodium chloride[2]	volume, %	140	147	165	121	122	126	122
	pH	6.55	6.80	6.75	6.50	6.70	6.65	6.50

[1] 1% $Na_4P_2O_7/Na_2H_2P_2O_7$-1.7% NaCl, pH 7.5, ionic strength, 0.66.
[2] 1.42% Na_2HPO_4/NaH_2PO_4-1.7% NaCl, pH 7.0, ionic strength, 0.66.

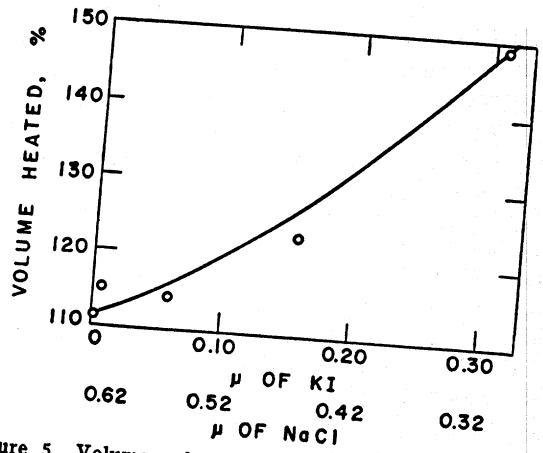

Figure 5. Volumes of meat samples treated with different proportions of NaCl and KI at constant ionic strength (μ) of 0.62.

In further investigating the effect of potassium iodide using Methods A-C and A-H, it was found that samples treated with solutions at pH 7.5 and ionic strength 0.67 and consisting of 1% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.7% NaCl; 1% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.69% NaCl and 0.017% KI; 1% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.64% NaCl and 0.17% KI; and 1% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.11% NaCl and 1.66% KI, had heated volumes of 129, 126, 127, and 167%, respectively. These results showed that treatment with solutions containing 1.66% KI markedly enhanced the retention of moisture as compared with pyrophosphate-sodium chloride while smaller amounts had no discernible effect. The contribution of potassium iodide to the total ionic strength, 0.67, of the solution was only 0.1 in this case. Similar results were obtained with NaI. The results point to the conclusion that the iodides produced an effect that the other additives produce only mildly, if at all, and thus was "specific." The addition of iodides produced no measurable effect on the pH of the meat; consequently, its effect could not be explained on the basis of its affecting either pH or ionic strength. KI has previously been found to increase the efficiency of muscle extraction, possessing, it is thought, a depolymerizing action on actin polymer and dissociating action on actomyosin *(14)*. While iodides have no practical use in sausage formulations, their pronounced effects indicate the possibilities which exist their mechanism of action can be duplicated with a chemical acceptable in food.

Effect of adding calcium and magnesium chlorides with other additives. In a series of experiments samples were treated with 1% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.7% NaCl, pH 7.5, and other samples with this solution plus 0.095% $MgCl_2$ or 0.11%, $CaCl_2$. Using steer beef which had been frozen for 4 weeks, the samples were treated by Methods A-C and A-H with the results shown in Table 2. It was found that the addition of $CaCl_2$, reduced and $MgCl_2$ increased meat volumes, the effects being consistent but not large. The results of Experiment 1, of Table 2, which was undertaken to obtain a statistical appraisal of these small differences, showed the increased volume obtained on adding $MgCl_2$ to be significant. In other experiments, the details of which are not reported, the two alkaline earth salts increased the moisture retention of samples treated with solutions of either sodium chloride or orthophosphate-sodium chloride to a similar extent.

The present results contrast, in part, with observations of previous workers. Bendall reported that the addition of 0.05M solutions of $MgCl_2$ had no effect on moisture retention, although he assumed that addition of the salt should improve the retention of moisture *(1)*. Hamm proposed that binding of calcium and magnesium are important functions of the phosphates in increasing the moisture retention of meat *(5)*. This appears inconsistent with the present results insofar as the effect of magnesium is concerned. Recognition of the number of known effects Mg has on muscle components and reactions of these components *(14)* precludes any present attempt to explain the beneficial effect of adding $MgCl_2$. Although the observed effects were not large, it tentatively appears that its addition to meat may have some practical value.

Experiments relating effectiveness to solubilization of proteins. The effect of treatments with pyrophosphate-sodium chloride solutions of different ionic strength and pH on meat volumes and, especially, on the solubility of proteins was investigated. Samples were treated with solutions of 1% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.7% NaCl, adjusted to pH values of 6.0, 7,25, and 8.5. The volumes (unheated) of the samples and the total and (acto)myosin nitrogen content of the solutions separated by centrifugation in following Method A-C were determined with the result shown in Table 3. The steer beef used had been frozen for 20 weeks. The nitrogen content of the precipitate formed on dilution of the supernatant liquid with 10 parts of water was considered to be (acto)myosin nitrogen.

Results show that volume of meat, concentration of nitrogen found in the supernatant liquid, and proportion of the dissolved nitrogen estimated to be (acto) myosin nitrogen increased as the pH and ionic strength of the applied solutions increased.

To evaluate the effect on meat volumes of heating

TABLE 2

Effect of adding $CaCl_2$ and $MgCl_2$ on volume of pyrophosphate-sodium chloride treated meat

Treatment	Volume, heated, %							
	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
$Na_4P_2O_7/Na_2H_2P_2O_7$-1.7% NaCl, pH 7.5	103[1]	(140)[2]	103	(6.50)[3]	108	(6.60)	129	(6.40)
95% $MgCl_2$ added to 1	106	(142)	110	(6.50)	113	(6.60)	134	(6.35)
65% $CaCl_2$ added to 1	.....		100	(6.40)	100	(6.50)	121	(6.40)

[1] Mean of six determinations, difference between 103 and 106% significant at the 10% level.
[2] Unheated volumes, means of six determinations, difference between 140 and 142% significant at the 5% level.
[3] pH values.

TABLE 3

Meat volume and solubility of proteins at varying pH and ionic strength

Treatment	Solution[1] pH	Volume unheated[2]	Nitrogen content of supernatant liquid	
			per ml.	(acto) myosin N_2
		%	*mg.*	%
1	6.0	128	5.88	18.9
2	7.25	149	6.35	37.6
3	8.5	179	7.41	39.8

[1] 1% $Na_4P_2O_7/Na_2H_2P_2O_7$-1.7% NaCl.
[2] % of original volume (20 ml.) of beef.

dissolved proteins, the effect of heating samples treated with pyrophosphate-sodium chloride solutions before, rather than after, centrifugation was determined. Solutions of 1% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.7% NaCl at pH values 5.6, 7.0, and 8.5 and ionic strength values 0.57, 0.66, and 0.73, respectively, were used. The meat used had been frozen for 24 weeks. Two procedures were applied: (1) Methods A-C and A-H, and (2) a modification of these in which samples were heated 15 minutes at 167° F. (75° C.) and then centrifuged 15 minutes, the usual order of these operations being reversed. Through comparison of the volumes (heated) obtained in following the two procedures, the effect of heating the solutions of proteins in contact with the meat mass was determined. The data obtained using the two methods are given in Table 4.

TABLE 4

Comparison of the effect on meat volume of heating meat-solution-mixtures before and after centrifuging

Treatment	Solution[1]		Volume of meat	
	pH	Ionic strength	Method A-H	Modified method[2]
			%	%
1	5.6	.57	100	
2	5.6	.57		123
3	7.0	.66	122	
4	7.0	.66		141
5	8.5	.73	153	
6	8.5	.73		178

[1] 1% $Na_4P_2O_7/Na_2H_2P_2O_7$-1.7% NaCl.
[2] Centrifugation, as in Method A-C, omitted; samples heated 15 minutes at 167° F. (75° C.) and centrifuged.

Results show that the volumes of samples which had been heated with all of the solutions containing dissolved proteins in contact with the meat were appreciably larger than the volumes of samples treated by Methods A-C and A-H, which were heated with only that solution intermixed with the meat which could not be separated by centrifugation in following Method A-C. From calculations based on the nitrogen content of the supernatant liquids (Table 3) and the data in Table 4, it was estimated that 1 g. of dissolved protein was responsible for retaining approximately 10 g. of moisture when heated in the presence of the meat mass.

Evaporation of moisture from treated samples heated 2 hours at 167° F. (75° C.). Inasmuch as the ordinary procedure in sausage making involves heating comminuted meat treated with no excess moisture and generally involves evaporation losses rather than separation of liquid fractions, Method B was applied to simulate practice in comparing the effect of treatments. A series of experiments was performed which afforded comparison of the evaporation of pairs of treatments, each experiment involving a comparison of the mean evaporation from 6 samples representing each of the two treatments. The solutions used, grouped in the pairs compared in individual experiments, were (1) no additive vs. 5% NaCl, (2) 5% NaCl vs. 1% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.7% NaCl, pH 8.5, (3) 1% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.7% NaCl, pH 7.0, vs. pH 8.5, (4) 1% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.7% NaCl, pH 7.5 vs. 1% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.11% NaCl–1.66% KI, pH 7.5, and (5) 1% $Na_4P_2O_7/Na_2H_2P_2O_7$– 1.7% NaCl, pH 7.5 vs. 1.42% Na_2HPO_4/NaH_2PO_4–1.87% NaCl, pH 7.5. The steer beef used had been frozen for 24-26 weeks. Significance of differences between means was determined with the "t" test. The results of the experiments are shown in Table 5. As indicated by the results, the order of effectiveness of treatments was that previously obtained by Methods A-C and A-H, *i.e.* orthophosphate-sodium chloride being more effective than pyrophosphate-sodium chloride at the same pH and ionic strength, the addition of KI increasing the effectiveness of pyrophosphate-sodium chloride, the pyrophosphates being more effective at higher pH, and pyrophosphates-sodium chloride being more effective than sodium chloride alone. The differences representing losses through evaporation were rather small. Since the exposed surface area of meat in the aluminum dishes had approximately the same relationship to the weight of

TABLE 5

Comparison of evaporation of moisture from samples held 2 hours at 167° F. (75° C.)

Number	Treatment	Differential evaporation		
		Evaporation[1]	Difference	Difference for 1% level
		%	%	%
1	Water	9.06		
	5% NaCl	8.30	0.76	0.73
	Water	9.50		
	5% NaCl	8.46	1.04	0.41
2	5% NaCl	8.95		
	1% $Na_4P_2O_7/Na_2H_2P_2O_7$– 1.7% NaCl, pH 8.5	8.23	0.72	0.53
	5% NaCl	8.85		
	1% $Na_4P_2O_7/Na_2H_2P_2O_7$– 1.7% NaCl, pH 8.5	8.41	0.44	0.35
	5% NaCl	9.63		
	1% $Na_4P_2O_7/Na_2H_2P_2O_7$– 1.7% NaCl, pH 8.5	8.71	0.92	0.55
3	1% $Na_4P_2O_7/Na_2H_2P_2O_7$– 1.7% NaCl, pH 7.0	9.22		
	1% $Na_4P_2O_7/Na_2H_2P_2O_7$– 1.7% NaCl, pH 8.5	8.66	0.56	0.50
	1% $Na_4P_2O_7/Na_2H_2P_2O_7$– 1.7% NaCl, pH 7.0	10.23		
	1% $Na_4P_2O_7/Na_2H_2P_2O_7$– 1.7% NaCl, pH 8.5	9.63	0.60	0.53
4	1% $Na_4P_2O_7/Na_2H_2P_2O_7$– 1.7% NaCl, pH 7.5[2]	9.86		
	1% $Na_4P_2O_7/Na_2H_2P_2O_7$–1.11% NaCl —1.66% KI, pH 7.5	9.11	0.75	0.35
5	1% $Na_4P_2O_7/Na_2H_2P_2O_7$– 1.7% NaCl, pH 7.5[2]	10.69		
	1.42% Na_2HPO_4/NaH_2PO_4– 1.87% NaCl, pH 7.5	9.70	0.99	0.43

[1] Mean evaporation of six samples.
[2] Ionic strength (μ) of each solution of pair was 0.67.

these samples as the surface of typical bologna has to its weight, it appears that only excellently controlled tests would prove that phosphate treatment reduced smokehouse losses in actual sausage-making operations.

SUMMARY

The moisture retention of lean meat treated with sodium chloride, pyrophosphate-sodium chloride, and other additives was investigated by a method involving the addition of solutions of salts in excess and measuring the amount of this excess after the samples were stored and centrifuged, and again after they were heated.

It was shown that unheated volumes were linearly related to heated volumes when samples were treated with sodium chloride, and curvilinearly related when samples were treated with pyrophosphate-sodium chloride.

It was found that the effectiveness of treatments with pyro- or orthophosphate-sodium chloride decreased as the temperature of application increased from 32 to 68° F. (0 to 20° C.). The effectiveness of treatments with sodium chloride was not similarly affected. These results emphasized the importance of maintaining low temperatures (*ca.* 32-41° F. or 0-5° C.) in using phosphate additives to obtain optimum moisture retention.

The rate at which sodium chloride and pyrophosphate-sodium chloride react with meat to produce their maximum effect was shown to be the same. The results showed that a nearly optimum effect was obtained after storage for 16 hours at 32° F. (0° C.).

Results show that the effectiveness of treatments with pyrophosphates was primarily related to the ionic strength and pH of the solutions applied. Aside from the pH of solutions, their capacity in buffering was shown to be important. Evidence failed to support a hypothesis that pyrophosphates exert an effect basically different from, or superior to, that of orthophosphates, at least under the conditions of these tests.

Addition of KI and NaI markedly enhanced moisture retention by exerting an effect different from, or markedly greater than, that of other additives and was thus "specific." Duplication of this mechanism with an acceptable food additive is a challenge.

Results showed that treatments with pyrophosphates dissolve proteins, especially (acto)myosin, to an extent affected by ionic strength and pH. Heating the dissolved protein in the presence of the meat markedly enhanced retention of moisture by the meat. Some of the factors influencing the effectiveness of various treatments were also found to affect the evaporation of moisture from samples heated at 167° F. (75° C.).

The findings are, in general, consistent with the premise that the factors governing the moisture retention of meat treated with phosphate additives are those which influence solubilization of muscle proteins; namely, temperature, time, ionic strength, and pH of treatments; and that specific effects can be exerted by ions, such as the I- and Mg^{++} ions.

LITERATURE CITED

1. Bendall, J. R. The swelling effect of polyphosphates on lean meat. *J. Science Food Agri.*, **5**, 468-475 (1954).
2. Ellerkamp, W., and Hannerland, H. Permissibility of polymerized phosphates in sausage meat. *Dtsch. Lebensmitt. Rdsch.*, **48**, 32-37 (1952).
3. Frank, Stefan S. Phosphates and the water-binding capacity of meats. (Thesis, Cornell University, 1955).
4. Grau, R., Hamm, R., and Bauman, A. The water-binding capacity of dead mammalian muscle. I. The effect of pH on water-binding of ground beef muscle. *Biochem. Z.*, **325**, 1-11 (1953).
5. Hamm, Reiner. The reason for the effect of additives and sodium chloride on meat. *Fleischwirtshaft*, **7**, 196-203 (1955).
6. Hasselbach, W., and Schneider, G. The L. myosin and and actin content of rabbit muscle. *Biochem. Z.*, **321**, 462-475 (1951).
7. Heil, G. Influence of phosphates on binding fat in cooked sausage. *Fleischwirtshaft*, **7**, 52 (1955).
8. Möhler, K., and Kiermeier, F. The effect of the addition of inorganic phosphate on animal proteins. I. The effect of the pH value of meats. *Z. Lebensm. -Untersuch. u. Forsch.*, **95**, 170-177 (1952).
9. Möhler, K., and Kiermeier, F. The effect of the addition of inorganic phosphates on animal proteins. II. Influence on the swelling of meat. *Z. Lebensm.-Untersuch. u. Forsch.*, **96**, 90-98 (1953).
10. Möhler, K., and Kiermeier, F. The effect of the addition of inorganic phosphates on animal proteins. III. Influence on the consistency of cooked meat. *Z. Lebensm.-Untersuch. u. Forsch.*, **97**, 100-105 (1953).
11. Möhler, K., and Kiermeier, F. The effect of the addition of inorganic phosphates on animal proteins. IV. The state of the pH value during the heat coagulation of meat protein. *Z. Lebensm.-Untersuch. u. Forsch*, **99**, 210-214 (1954).
12. Mommaerts, W. F. H. M. The reaction between actomyosin and adenosine triphosphate. *J. Genl. Physiol.*, **31**, 361-375 (1948).
13. Morse, Roy. How phosphates can benefit meats. *Food Eng.*, **27**, 84-86 (1955).
14. Neurath, H., and Bailey, K. *The Proteins.* 1954, Vol. II, Part B, Chapter 24, Academic Press Inc., New York, N. Y.
15. Straub, F. B. On the specificity of the ATP-effect. Studkes Inst. Med. Chem., Univ. Szeged, Vol. III, 39 (1943).
16. Swift, C. E., Weir, C. E., and Hankins, O. G. The effect of variation in moisture and fat content on the juiciness and tenderness of bologna. *Food Technol.*, **8**, 339-340 (1954).
17. Swift, C. E., and Hankins, O. G. Variance in the chemical determination of added moisture. *Food Technol.*, **8**, 323-325 (1954).
18. USDA Meat Inspection Branch Memo. No. 190.
19. Wilson, G. D. Additives to sausage and cured meats. *National Provisioner*, (Oct.) 112 (1954).